7. Barron, E. J., and Hanahan, D. J., J. Biol. Chem. 231, 493 (1958).

8. Chang, R. S., Proc. Soc. Exptl. Biol. Med. 87, 440 (1954).

9. McQuilkin, W. T., Evans, V. J., and Earle, W. R., J. Natl. Cancer Inst. 19, 855 (1957).

10. Fisher, R. A., "Statistical Methods for Research Workers," 13th ed. Hafner, New York, 1967.

11. Mackenzie, C. G., Mackenzie, J. B., and Reiss, O. K., J. Lipid Res. 8, 642 (1967).

12. Mackenzie, C. G., Mackenzie, J. B., and Reiss, O. K., in "Lipid Metabolism in Tissue Culture Cells," Rothblat, G. H. and Kritchevsky, D., eds., p.63. The Wistar Symposium Monograph No. 6, Wistar Inst. Press, Philadelphia, Pennsylvania, 1967.

13. Bailey, J. M., Biochim. Biophys. Acta 125, 226 (1966).

14. LeFevre, P. G., in "Protoplasmatologia," Heilbrunn, L. V. and Weber, F., eds., Vol. 8, No. 7a, p.1. Springer, Vienna, 1955.

15. Rothblat, G. H., Martak, D. S., and Kritchevsky, D., Proc. Soc. Exptl. Biol. Med. 112, 598 (1963).

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Intensive Nursing and Lactational Performance during Extended Lactation* (32939)

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Suckling stimulation and milk removal are important in maintenance of mammary structure and milk synthesis during lactation. Adjusting litter size of rats on the third day of lactation to six pups per six (6/6) intact abdominal-inguinal mammary glands caused significant progressive increases in mammary deoxyribonucleic acid (DNA) content to day 16 and in ribonucleic acid (RNA) to day 20 of lactation (1). However, the 6/6 suckling intensity of the original litter was not sufficiently strong to prevent eventual declines in both DNA and RNA (1). Fostering litters of rats has been used to extend lactation beyond its normal length (2,3), but maintenance of maximal milk secretion rates has not been achieved. Possible ways of providing a maximal nursing stimulus for maintenance of lactation which have not been investigated in previous studies are: frequent replacement of litters; use of litters 12 to 16 days of age (when nursing intensity is greatest); and maintenance of at least one pup per mammary gland. The objective of this experiment was to determine if intensive nursing would maintain mammary cell numbers (DNA) and secretory activity (RNA, RNA/DNA, and litter weight gain) during an extended lactation. A second objective was to relate changes in mammary cell numbers and synthetic activity with prolactin and adrenocorticotropic hormone (ACTH) contents in the pituitary, two hormones that have been implicated in regulation of lactation (4).

Materials and Methods. On the third day of lactation thoracic teats of 48 Sprague-Dawley rats were ligated, litter size adjusted to 6 pups, and mother rats assigned to one of four groups to be killed either on day 16, 20, 28, or 36 of lactation. Both 16-day-old original litters and foster litters were replaced every 4 days with 12-day-old foster litters. Cumulative litter weight gains were recorded between days 13 and 16 of age for all litters. Final body weights of lactating rats were recorded at time of killing, and nucleic acid content determined on 6 abdominal-inguinal mammary glands as previously described (5).

Twelve anterior pituitaries were collected from each of 2 additional groups of rats killed on either day 20 or 36 of lactation. Prolactin potency of the 12 pooled pituitaries of each group was estimated by the method of Reece and Turner (6). The ACTH potency of the 2 groups of pituitaries was determined by the method of Saffran and Schally (7). Prolactin

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TABLE I. Nucleic Acid Content of Mammary Glands and Litter Weight Gain of Intensely[a] Suckled Rats during Extended Lactation.

	Days of lactation[b,c]			
	16	20	28	36
Body wt. (gm)	242 ± 5.2	253 ± 6.0	259 ± 2.8	273 ± 3.8
Fresh gland wt. (gm)	12.00 ± .68	12.94 ± .56	10.62 ± .41	11.16 ± .42
Total DNA (mg)	36.2 ± 1.4	37.2 ± 1.2	32.0 ± .9	27.0 ± .2
Total RNA (mg)	206.7 ± 8.9	226.5 ± 9.9	154.4 ±19.9	104.3 ± 6.2
RNA/DNA	5.7 ± .1	6.1 ± .1	4.8 ± .2	3.8 ± .2
Litter weight gain (gm)[d]	33.5 ± 2.1	39.3 ± 1.8	20.8 ± 2.6	3.9 ± 1.5

[a] Litters 16 days old replaced with 12-day-old foster litters.

[b] Mean and SE of mean.

[c] See results in text for significant differences among means.

[d] Cumulative litter weight gains were recorded between days 13 and 16 of age for all litters.

standard was NIH-P-S6 prolactin[1] and ACTH standard was porcine ACTH from Mann Research Laboratories, Inc., New York, N. Y. We have recently described procedural details of both assays (8). Litter weight gains and nucleic acid content of mammary tissue of these two groups were determined as above. In addition, lactic acid content of mammary glands was determined (9) as another index of mammary involution (4).

Results. Mammary weights at days 16 and 20 of lactation were greater ($p<0.01$) than at days 28 and 36 (Table I) but weights were not significantly different ($p>0.05$) between day 16 and 20 nor between day 28 and 36. Total mammary DNA did not change significantly ($p>0.05$) between days 16 (36.2 mg) and 20 (37.2 mg). However, mammary DNA declined linearly ($p<0.01$) between days 20 and 36 (27.0 mg) of lactation. Mammary RNA did not change significantly ($p=0.10$) between days 16 (206.7 mg) and 20 (226.5 mg), but a linear decline ($p<0.01$) of 54% was observed between day 20 and day 36. Mammary RNA/DNA ratios were 5.7, 6.1, 4.8, and 3.8 for groups killed on days 16, 20, 28, and 36, respectively. Thus, changes in ratio paralleled very closely total RNA changes. Since body weight of mother rats increased progressively during lactation ($p<0.01$), correcting for body weight merely magnifies the difference among groups for nucleic acid changes. Cumulative litter weight gains for the 3-day period prior to sacrifice at 16, 20, 28, and 36 days of lactation were 33.5, 39.3, 20.8, and 3.9 gm, respectively. The overall correlation between total RNA and litter weight gain was 0.88 ($p<0.01$).

In the second experiment mammary DNA, RNA, RNA/DNA, and 3-day cumulative litter weight gains declined 17.5, 52.9, 43.0, and 113.9%, respectively, between days 20 and 36 of lactation. These declines were in close agreement with declines observed in the initial experiment. Pituitaries of rats killed at days 20 and 36 of lactation contained .018 and .015 IU of prolactin/mg of pituitary, respectively, and 22.5 and 7.3 milliunits of ACTH/mg of pituitary, respectively (Table II). Total lactic acid content of mammary glands was 5.9±.3 and 5.0±.4 mg for the 20- and 36-day treatment groups, respectively. There was no significant difference ($p>0.05$) between mammary gland weights; therefore, lactic acid content per gram of mammary tissue was not significantly different between the two groups ($p>0.05$).

Discussion. Frequent replacement of foster litters to maintain intensive nursing stimulation failed to prevent declines in mammary DNA, RNA, RNA/DNA, and cumulative litter weight gain between days 20 and 36 of lactation. The high correlation between litter weight gain and mammary RNA content confirmed previous experiments (1,5). Losses of RNA during extended lactation were much greater than losses in DNA, and suggested

[1] Ovine prolactin (NIH-P-S6) was supplied by Natl. Inst. Health.

TABLE II. Prolactin and ACTH Content of Pituitaries of Intensely[a] Suckled Rats during Extended Lactation.

Days of lactation	Av pituitary wt. (mg)	Prolactin (IU/mg)				ACTH (milliunits/mg)			
		Potency	SE	CI 95%	λ	Potency	SE	CI 95%	λ
20	9.0	.018	.011	.004–.037	.40	22.5	9.4	5.7–84.8	.21
36	9.2	.015	.008	.004–.030	.36	7.3	1.0	5.0–10.8	.08

[a] Litters 16 days old replaced with 12-day-old foster litters.

that factors controlling protein synthesis limited milk synthesis more than factors influencing cell numbers. Support for this concept was provided by Tucker and Reece (3) who replaced foster litters much less frequently than was done in the present study and showed no significant changes in DNA but marked declines in RNA and litter weight gain during extended lactation.

Lactic acid production increases in mammary glands when milk is not removed, but no increases occur when milk is removed (4). The similar lactic acid content of mammary glands from rats sacrificed at 20 or 36 days of lactation suggested that frequent litter exchanges permitted adequate milk removal. These results further suggested that increased anaerobic oxidation was not a limiting factor to milk synthesis during extended lactation.

Previous results indicated that pituitary prolactin potency increased with increases in suckling stimulation, but ACTH did not change significantly up to day 16 of lactation (8,10). In the present experiments intense nursing maintained pituitary prolactin content at least until day 36 of lactation. However, intense nursing failed to prevent a decline of 67.6% in pituitary ACTH between days 20 and 36 of lactation. This decline in ACTH during extended lactation confirmed similar observations made between the first and sixteenth day of lactation (10). Furthermore, the declines in ACTH paralleled declines observed in mammary nucleic acid content and litter weight gain during extended lactation. If reduced pituitary ACTH content reflects an actual decline in synthesis of the hormone, ACTH may be one of the hormonal factors that limits milk synthesis during extended lactation.

Summary. Intensive nursing (6 pups/6 intact abdominal-inguinal mammary glands, and replacing all 16-day-old litters every 4 days with 12-day-old foster litters) failed to prevent significant declines in mammary DNA, RNA, and litter weight gains between days 20 and 36 of lactation. But no comparable changes in lactic acid content of mammary glands were observed. Intense nursing maintained pituitary prolactin during extended lactation but ACTH decreased 67.6%. Thus, ACTH may be rate limiting to milk synthesis during extended lactation.

1. Tucker, H. A., Am. J. Physiol. **210**, 1209 (1966).

2. Nicoll, C. S. and Meties, J., Proc. Soc. Exptl. Biol. Med. **101**, 81 (1959).

3. Tucker, H. A. and Reece, R. P., Proc. Soc. Exptl. Biol. Med. **112**, 668 (1963).

4. Cowie, A. T. and Folley, S. J., in "Sex and Internal Secretions," Young, W. C., ed. Vol. 1, pp. 598, 610, Williams and Wilkins, Baltimore, Maryland (1961).

5. Tucker, H. A., Proc. Soc. Exptl. Biol. Med. **116**, 218 (1964).

6. Reece, R. P. and Turner, C. W., Missouri Univ. Agr. Exptl. Sta., Res. Bull. **266** (1937).

7. Saffran M. and Schally, A. V., Endocrinology **56**, 523 (1955).

8. Tucker, H. A. Paape, M. J., Sinha, Y. N., Pritchard, D. E., and Thatcher, W. W., Proc. Soc. Exptl. Biol. Med. **126**, 100 (1967).

9. Barker, S. B. and Summerson W. H., J. Biol. Chem. **138**, 135 (1941).

10. Tucker, H. A., Paape, M. J. and Sinha, Y. N., Am. J. Physiol. **213**, 262 (1967).

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